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13. ABSTRACT (Maximum 200 Words)

Chemokines play a pivotal role in the maturation of the immune system, and in the initiation, and maintenance of an immune response. Because of their key role in the immune response, the aberrant expression of chemokines can have a profound effect on the ability of T cells to respond to antigen. We have found that several breast cancer cell lines produced chemokines capable of recruiting T cells. However, instead of increasing anti-tumor immunity, the tumor-derived chemokines may have prevented an effective immune response by desensitizing T-cell chemokine receptors. Our hypothesis is that disrupting the synthesis of tumor-derived chemokines (using anti-sense technology) will remove tumor-induced immune suppression and enhance the immunogenicity of the tumor. In order to test this hypothesis we have generated stable clones that lack MCP-1 and RANTES production compared to the parental tumor cell line. Using these tumors we have found that both MCP-1 and RANTES impair the T cell response to the murine mammary carcinoma 4T1. Tumors that lack MCP-1 or RANTES elicited a better tumor-specific T cell response. Studies are underway to determine the extent of tumor immunity generated to a tumor that lacks both of these chemokines.

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Table of Contents

INTRODUCTION

Chemokines play a pivotal role in the maturation of the immune system, and in the initiation, and maintenance of an immune response (1). Because of their key role in the immune response, aberrant expression of chemokines can have a profound effect on the ability of T cells to respond to antigen. We have found that several breast cancer cell lines produced chemokines [Regulated upon activation, normal T cell expressed and secreted (RANTES) and monocyte chemotactic factor-1 (MCP-1)] capable of recruiting T cells, as well as the chemokine KC (2). Additionally, supernatants derived from the tumor cell line 4T1 could mediate the chemotaxis of T cells. However, instead of increasing anti-tumor immunity, the tumor-derived chemokines may have prevented an effective immune response by desensitizing T-cell chemokine receptors (2). The receptors for RANTES and MCP-1 on T cells were desensitized in tumor-bearing animals. Moreover, there was cross-receptor desensitization of the CC chemokine receptor 7 (CCR7), which impaired the ability of the T cells to respond to secondary lymphoid chemokine, SLC. These data indicate that the aberrant expression of tumor-derived chemokines may help tumors escape immune attack. Our hypothesis is that disrupting synthesis of tumor-derived chemokines (using anti-sense technology) will remove tumor-induced immune suppression and enhance the immunogenicity of the tumor. We will determine whether the T cells are better able to elicit an anti-tumor immune response by comparing the immunogenicity of the tumors that do and do not express chemokines. These tumor cells will be evaluated by immunization/challenge experiments and by the ability to generate tumor-specific T cells in tumor draining lymph nodes.

BODY

The first objective of the project was to generate sense and anti-sense constructs capable of inhibiting synthesis of the tumor-derived chemokines RANTES, MCP-1, and KC. This objective has been completed. The second and third objectives of the proposal (months 10-36) are underway as described below. Transfection of the 4T1-9 tumor cell line has been accomplished with the sense and anti-sense RANTES, MCP-1 and KC vectors. Progress with each chemokine is described separately below.

Statement of Work

Objective 1. Generate anti-sense constructs capable of inhibiting synthesis of tumor-derived chemokines, months 1-9:

a. PCR amplify RANTES, MCP-1 and KC from the murine breast cancer cell line 4T1.
b. Ligate the chemokines into the T-vector.
c. Transform the vector containing the chemokines into competent E. coli.
d. Screen for clones that contain the correct insert by blue/white screening and a BamH1/Not1 restriction digest.
e. Digest the correct clones with BamH1 and Not1 and gel purify the chemokine DNA.
f. Separately ligate each chemokine into the vector in the reverse orientation.

milestone #1-Single antisense vectors constructed [completed]

g. Ligate together the RANTES, MCP-1, and KC PCR products.
h. Transform the vector containing all three chemokines into competent E. coli.
i. Screen for the clone that contains the correct insert by blue/white screening and a BamH1/Not1 restriction digest.
j. Digest the correct clone with BamH1 and Not1 and gel purify the chemokine DNA.
k. Ligate the chemokine DNA into the vector in the reverse orientation for the triple antisense construct.

milestone #2-Triple antisense vector constructed

*Objective 2.*Transduce and clone tumor cells that lack production of chemokines, months 10-18:

a. Package the retroviral construct by transfecting the PA317 packing cell line.
b. Collect, concentrate and titre the virions.
c. Transduce 4T1 with each construct.
d. Drug selection and reclone the tumor cells.
e. Screen the transduced clones by RT-PCR for the presence of the antisense construct, and chemokine mRNA synthesis.
f. Screen the transduced clones by ELISA for chemokine protein synthesis.
g. Screen the supernatants from the transduced clones for T cell chemotactic ability.

milestone #3-Tumor cells cloned with antisense transgenes. [completed]

Objective 3. Examine the ability of tumor cells lacking chemokines to induce chemokine receptor desensitization and for increased immunogenicity, months 19-36:

a. Compare desensitization of chemokine receptors on T cells from 4T1 tumor- bearing mice to T cells from the transduced 4T1 tumor-bearing mice. We will perform this assay three times with each of the anti-sense expressing clones.
b. Compare the immunogenicity of the clones by immunization/challenge experiments.
c. Compare the immunogenicity of the clones by determining their ability to generate tumor-specific T cells in vaccine draining lymph nodes.

milestone #4-Evaluate whether targeted disruption of tumor-derived chemokine synthesis reverses tumor-induced immune suppression. [completed for RANTES, completed for MCP-1]

Impact of tumor-derived RANTES

RANTES is constitutively expressed by the 4T1 tumor cell line as well as T cells, epithelial cells and platelets following exposure to inflammatory agents or mitogens (1, 2). Mast cells, T cells, natural killer cells, dendritic cells, eosinophils and basophils are capable of responding to RANTES via CCR1, CCR2, CCR3, CCR4 and/or CCR10 (3). In order to study the impact of tumor-derived RANTES on anti-tumor immunity we attempted to inhibit RANTES production using anti-sense technology. For this purpose the tumor cells were transfected with the sense and anti-sense vectors, cloned and screened for RANTES production by RT-PCR and ELISA. Sense (R1) and anti-sense (RA5) clones were selected, expanded and supernatants were taken at 1, 4, 8 and 24 hours in order to examine the kinetics of RANTES production (Figure 1A). The data revealed that in three separate experiments RANTES production was consistently normal in the sense clone and the anti-sense clone consistently expressed ten-fold less RANTES. In order to examine the stability of the clones RANTES production was examined after the clones were grown in vivo for 4 weeks in the absence of G418 (Figure 1B). These data verified the stability of the 4T1 clones that expressed significantly less RANTES than the parental 4T1 and the vector transfected control cells (R1).

The in vitro and in vivo growth rates were analyzed and revealed that although the clones grew at similar rates in vitro, the anti-sense clone grew slower in vivo than the sense clone (Figure 2). In order to determine whether a T cell response was associated with the decreased growth rate of the RA5 tumor we first examined tumor-infiltrating lymphocytes (TIL). We found that the tumor that produced less RANTES had a greater T cell infiltrate than the control cells (Figure 3A). Moreover, the greater lymphocytic infiltrate was shown to be CD3/CD4 and CD3/CD8 + T cells (Figure 3B). The greater infiltration of the tumors by the lymphocytes was found to coincide with the prevention of altered T cell chemotactic activity we previously reported (2). When splenocytes from tumor-bearing mice were compared we found that T cells from RA5 tumor-bearing mice were able to migrate as well as T cells from naïve mice (Figure 4). Thus, blockade of RANTES production prevented the tumor-induced alteration in T cell chemotactic activity. In order to determine whether a T cell response coincided with normal migratory activity we examined T cells from tumor draining lymph nodes (Figure 5). These data showed that the RA5 tumor elicited a better tumor-specific response than the R1 tumor. Finally, we performed vaccination/challenge experiments and found that despite the enhanced T cell response the RA5 tumor was not more immunogenic based upon the vaccination/challenge experiments (Figure 6). These experiments complete the RANTES portion of the project.

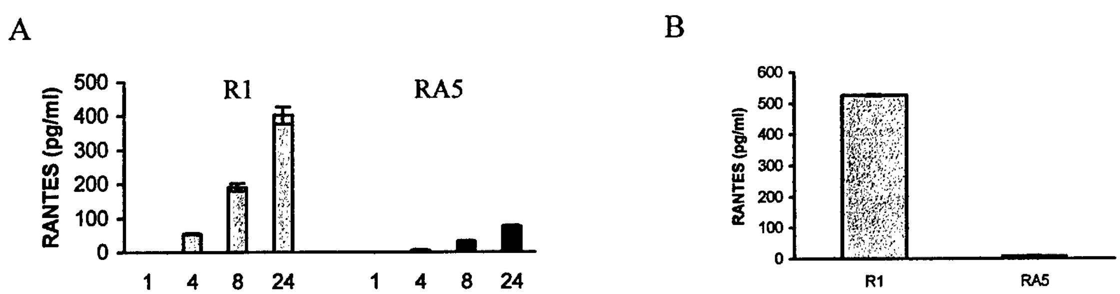

Figure 1. Kinetics and stability of RANTES production. A. The sense transfected clone (R1) and anti-sense clone (RA5) were plated at $1x10^6$ cells/well in a 24 well plate and supernatants were harvested at 1, 4, 8 and 24 hours and analyzed for RANTES by ELISA. The experiment

was repeated three times with similar results. Error bars represent standard deviation of duplicate samples. B. The sense and anti-sense clones were injected into Balb/c mice and recovered after 30 days of tumor growth. The tumors were digested in collagenase cocktail and plated in cRPMI. After 3 days the tumor cells were harvested and plated at $1x10^6$ cells/well in a 24 well plate. Twenty-four hours later the supernatants were harvested and analyzed for RANTES by ELISA. The results are representative of three separate experiments with the standard deviation of duplicate samples shown.

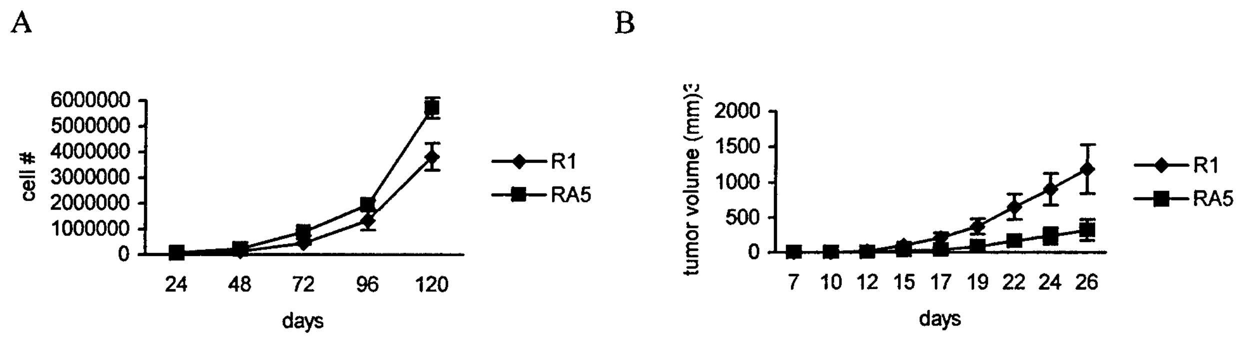

Figure 2. Growth kinetics of the RANTES clones. A. In order to examine the in vitro growth rate of the RANTES sense and anti-sense clones $2x10^4$ cells were plated in tissue culture flasks, collected at 24-120 hours and counted. The experiment was repeated three times with similar results. B. In order to examine the in vivo growth rates of the sense and anti-sense clones $5x10^4$ cells were delivered subcutaneously (sc) to each mouse and the tumors were measured for 26 days. The average of 5 mice is shown. The experiment was performed three times with similar results.

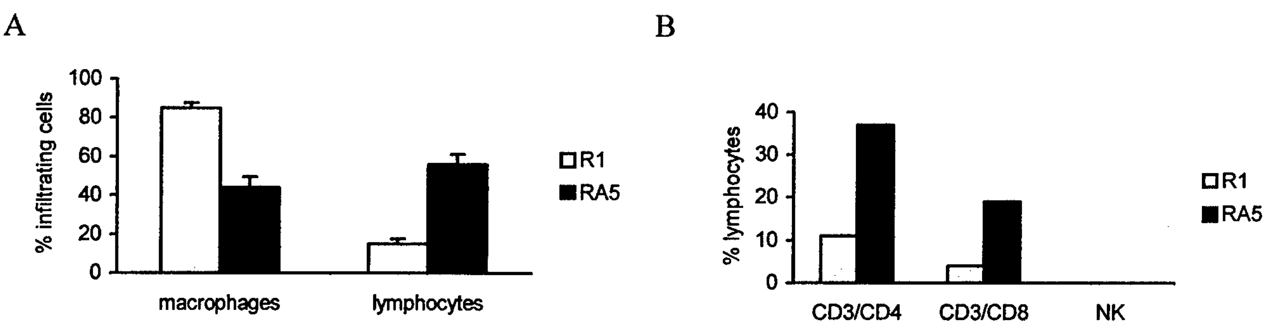

Figure 3. TIL analysis. Following 28 days of tumor growth the R1 and RA5 tumors were digested in a collagenase cocktail. A. A differential cell count revealed a greater lymphocyte infiltration into the RA5 compared to the R1 tumors. The results are representative of three separate experiments. B. The lymphocytes were phenotyped in order to determine the identity of the infiltrating cells. The data are representative of two separate experiments.

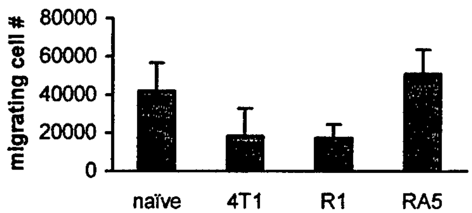

Figure 4. Splenocyte chemotactic activity. Splenocytes were harvested from naïve, 4T1, R1 and RA5 tumor-bearing mice and assessed for chemotactic activity in a one-hour chemotaxis assay. The data revealed that blockade of tumor-derived RANTES prevented the altered chemotactic activity in tumor-bearing mice. The data were averaged from three separate experiments with the standard error shown.

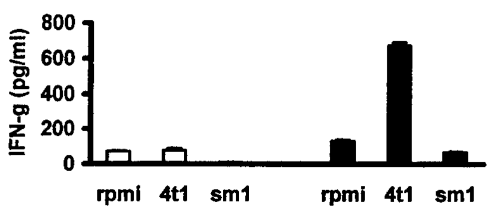

Figure 5. Analysis of T cells from tumor-draining lymph nodes. Eight mice were given R1 (□) or RA5 (■) tumors and eight days later the tumor-draining lymph nodes were harvested. The recently activated $CD62L^{lo}$ cells were isolated by magnetic cell separation and expanded with antibody to CD3 and IL-2. The T cells were harvested and a cytokine release assay was used to assess reactivity to the 4T1 tumor cell line or the syngeneic murine mammary carcinoma SM1. The data are representative of four separate experiments.

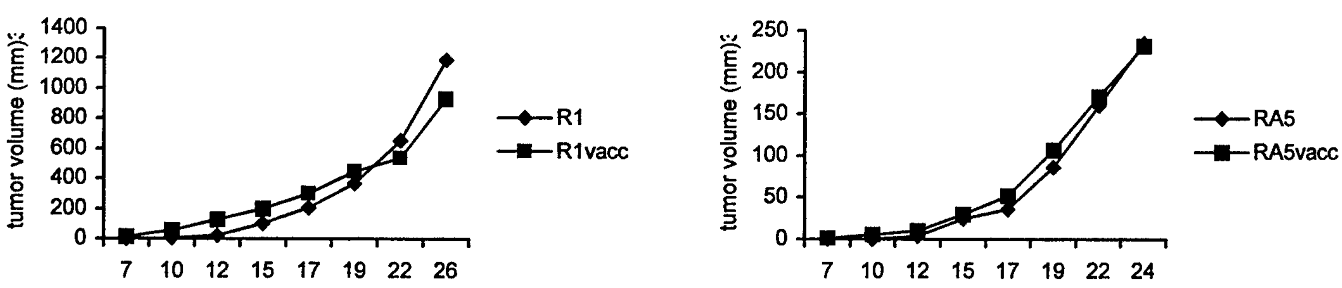

Figure 6. Analysis of tumor immunogenicity. Vaccination/challenge experiments were used to determine whether blockade of RANTES production would increase the immunogenicity of the tumor cells. For this purpose 5-10 mice were given $1x10^7$ mitomycin-C treated R1 or RA5

tumor cells and fourteen days later challenged with $5x10^4$ tumor cells. The data are representative of three separate experiments.

Summary of findings with RANTES

1. Anti-sense strategy can effectively block tumor-derived RANTES production.
2. Tumor-derived RANTES increases the in vivo growth rate of 4T1 murine mammary carcinoma.
3. Tumor-derived RANTES inhibits the T cell response to 4T1 murine mammary carcinoma.
4. Tumor-derived RANTES alters T cell chemotactic activity in tumor-bearing mice.
5. Tumor-derived RANTES impairs TIL recruitment to the tumor.

These data are being prepared for submission. We anticipate submitting the manuscript to the International Journal of Cancer by the end of summer (2003).

Impact of tumor-derived MCP-1

MCP-1 is constitutively expressed by the 4T1 tumor cell line as well as macrophages, neutrophils, mast cells and fibroblasts during an inflammatory response (1, 2). Macrophages, dendritic cells and T cells are capable of responding to MCP-1 via CCR2 and/or CCR4 (3). In order to study the impact of tumor-derived MCP-1 we attempted to inhibit MCP-1 production using anti-sense technology. For this purpose tumor cells were transfected with sense and anti-sense vectors, cloned and screened for MCP-1 production by RT-PCR and ELISA (figure 7). ELISA revealed a clone (G7) that did not produce detectable levels (assay sensitivity <15pg/ml) of MCP-1. The sense (A4) and anti-sense (G7) expressing clones were expanded and supernatants were taken at 1, 4, 8 and 24 hours in order to examine the kinetics of MCP-1 production (Figure 7A). The data revealed that in three separate experiments MCP-1 production was consistently normal in the sense clone and consistently below detection in the anti-sense clone. In order to examine stability of MCP-1 production we examined levels after the clones were grown in vivo for 21 days in the absence of G418 (Figure 7B). The data show that production of MCP-1 by the A4 clone and lack of production by the G7 clone was stable.

Next we examined the in vitro and in vivo growth rates of the tumor cells. The data showed that modulation of tumor-derived MCP-1 did not alter the in vitro or in vivo growth rates of the tumor cells (Figure 8). Although we found blockade of MCP-1 production did not prevent the altered chemotactic activity of splenic T cells (data not shown), we did find that the T cell response was enhanced (Figure 9). A cytokine release assay using $CD62L^{lo}$ cells from tumor-draining lymph nodes showed that T cells draining the G7 tumor produced more IFN-γ in response to the tumor than T cells draining the A4 tumors. We also found that the decrease in IFN-γ production did not coincide with an increase in IL-4 production (Figure 9B). Therefore, tumor-derived MCP-1 decreased the ability of T cells to make IFN-γ in response to the tumor cells and did not induce a Th1 to Th2 switch. Analysis of the TCR Vβ usage showed that the same T cells predominated both sets of lymph nodes (Figure 10). Therefore, we investigated whether MCP-1 could directly modulate T cell cytokine production. We were able to show that recombinant MCP-1 elicited the same response in vitro. T cells incubated with recombinant MCP-1 produced less IFN-γ in response to stimulation with antibody to CD3 (Figure 11). Thus, this tumor-derived chemokine appears to directly impair the ability of T cells to make IFN-γ. As a result of these findings we expected that the G7 tumors would be more immunogenic in the vaccination/challenge experiments. However, we found that modulation of tumor-derived MCP-1 did not alter the immunogenicity of the tumor cells despite the alteration in T cell effector function (Figure 12). These data indicated that tumor-derived MCP-1 could impair T cell effector function, but not enough to modulate the immunogenicity of the tumor cells. These, experiments complete the MCP-1 portion of the project.

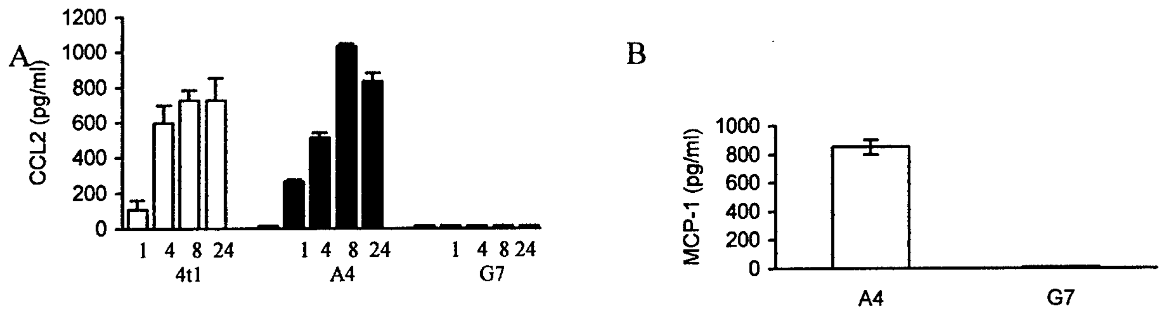

Figure 7. Kinetics and stability of MCP-1 production. A. The parental tumor (4T1), sense (A4) and anti-sense clones (G7) were plated at $1x10^6$ cells/well in a 24 well plate and supernatants were harvested at 1, 4, 8 and 24 hours and analyzed for MCP-1 by ELISA. The experiment was repeated three times with similar results. Error bars represent standard deviation of duplicate samples. B. The sense and anti-sense clones were injected into Balb/c mice and recovered after 21 days of tumor growth. The tumors were digested in collagenase cocktail and plated in cRPMI. After 7 days the tumor cells were harvested and plated at $1x10^6$ cells/well in a 24 well plate. Twenty-four hours later supernatants were harvested and analyzed for MCP-1 by ELISA. The results are representative of three separate experiments with the standard deviation of duplicate samples shown.

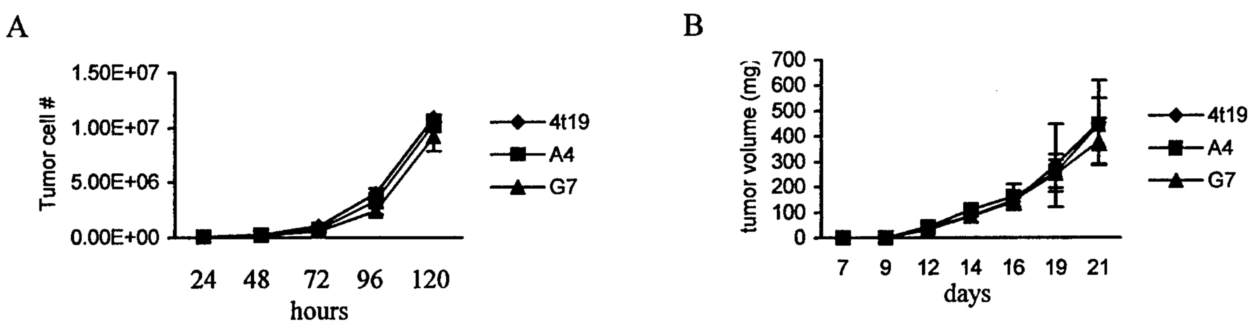

Figure 8. Growth kinetics of the MCP-1 clones. A. In order to examine the in vitro growth rate of the MCP-1 sense and anti-sense clones $2x10^4$ cells were plated in tissue culture flasks, collected at 24-120 hours and counted. The experiment was repeated three times with similar results. B. In order to examine the in vivo growth rates of the sense and anti-sense clones $5x10^4$ cells were delivered sc to each mouse and the tumors were measured for 21 days. The average of 5 mice/group is shown. The experiment was performed three times with similar results. Parental tumor cells (4T1) were used as controls.

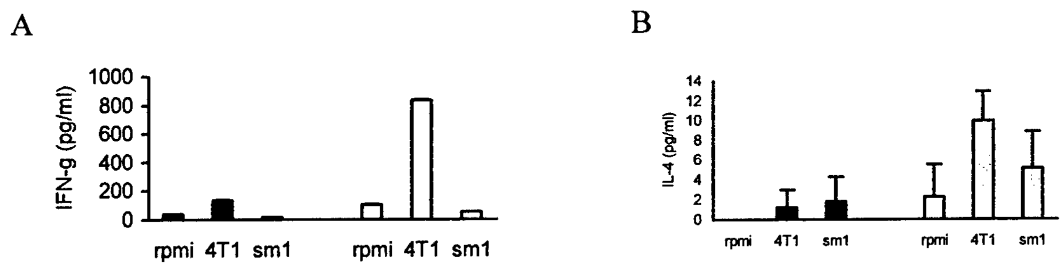

Figure 9. Analysis of T cells from tumor-draining lymph nodes. Eight mice were given A4 (■) or G7 (□) tumors and eight days later the tumor-draining lymph nodes were harvested. The recently activated CD62L^{lo} cells were isolated by magnetic cell separation and expanded with antibody to CD3 and IL-2. The T cells were harvested and a cytokine release assay used to assess reactivity to the 4T1 tumor cell line or the syngeneic murine mammary carcinoma SM1 with an IFN-γ (A) and IL-4 (B) specific ELISA. The data are representative of three separate experiments.

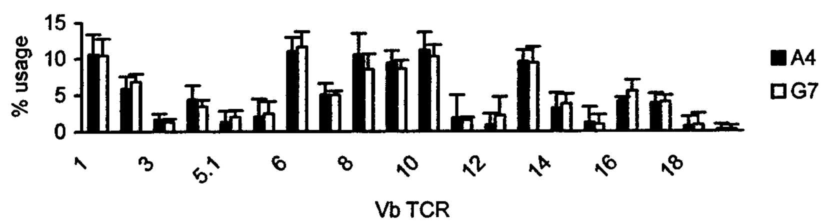

Figure 10. VβTCR usage in tumor-draining lymph nodes. To determine whether modulation of tumor-derived MCP-1 altered the T cell response to the tumor we examined the VβTCR usage of the CD62L^{lo} cells found in the tumor-draining lymph nodes. For this purpose mRNA was isolated from the T cells, converted to cDNA and semi-quantitative RT-PCR was used to compare VβTCR usage. The data revealed no major differences in T cell response to the A4 and G7 tumors. The data are representative of three separate experiments.

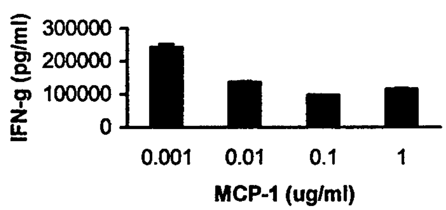

Figure 11. Recombinant MCP-1 modulates T cell IFN-γ production. To determine whether MCP-1 could directly modulate the ability of T cells to produce IFN-γ we incubate T cells with recombinant MCP-1 for 72 hours and then stimulated the cells with antibody to CD3. After 24 hours supernatants were harvested and assessed for IFN-γ production. The data are representative of three separate experiments.

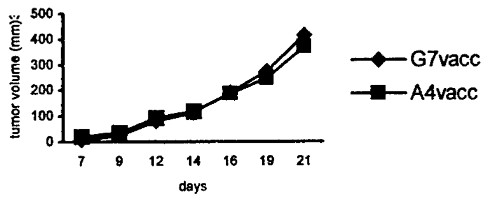

Figure 12. Analysis of tumor immunogenicity. Vaccination/challenge experiments were used to determine whether blockade of MCP-1 production would increase the immunogenicity of the tumor cells. For this purpose 5-10 mice were given $1x10^7$ mitomycin-C treated A4 or G7 tumor cells and fourteen days later challenged with $5x10^4$ tumor cells. The data are representative of three separate experiments.

Summary of findings with MCP-1

1. Anti-sense strategy can effectively block tumor-derived MCP-1 production.
2. Blockade of tumor-derived MCP-1 does not alter the growth kinetics of the 4T1 murine mammary carcinoma.
3. Blockade of tumor-derived MCP-1 can increase the ability of T cells to produce tumor-specific IFN-γ.
4. MCP-1 can directly modulate the ability of T cells to produce IFN-γ.
5. Modulation of tumor-derived MCP-1 did not alter the immunogenicity of the 4T1 murine mammary carcinoma.

These data were submitted to Immunological Letters for publication on 5/13/2003 (4).

Impact of tumor-derived KC

KC is constitutively expressed by the 4T1 tumor cell line as well as neutrophils, epithelial cells and platelets following exposure to inflammatory agents or mitogens (1, 2). Mast cells, neutrophils, eosinophils and basophils are capable of responding to KC via CXCR2, and to a lesser extent CXCR1 (3). In order to study the impact of tumor-derived KC we attempted to inhibit KC production using anti-sense technology. For this purpose the tumor cells were transfected with sense and anti-sense eukaryotic expression vectors, cloned and screened for KC production by RT-PCR and ELISA. Using two different vectors we transfected and screened over 300 clones and found approximately 20 clones that had no or low levels of KC expression (Figure 13A). However, after the clones were expanded and the kinetics of KC production was examined the clones were found to have regained KC expression (Figure 13B). Of significant interest we observed that the clones that initially expressed the lowest levels of KC grew the slowest in culture and over time the growth rate increased as well as KC expression. Thus, it appeared that KC production was associated with cell growth rate. Previously, it has been reported that the human KC equivalent Gro acts as an autocrine growth factor for human melanoma (5-7). Therefore, we began to investigate whether KC functioned as an autocrine growth factor for the murine mammary carcinoma 4T1. If KC were an autocrine growth factor it would explain the difficulty in generating a KC (-) tumor cell line. For this purpose we assessed whether the receptor for KC was present on the tumor cells. We designed primers for CXCR1 and CXCR2 and found that 4T1 cells expressed both receptors (Figure 14). We also evaluated whether neutralization of KC using a monoclonal antibody could slow the growth of the tumor cells in vitro. Preliminary data showed that neutralization of KC could modulate tumor growth in vitro in a dose dependent manner (Figure 15). Thus, we have preliminary evidence that KC may function as an autocrine growth factor for the murine mammary carcinoma 4T1.

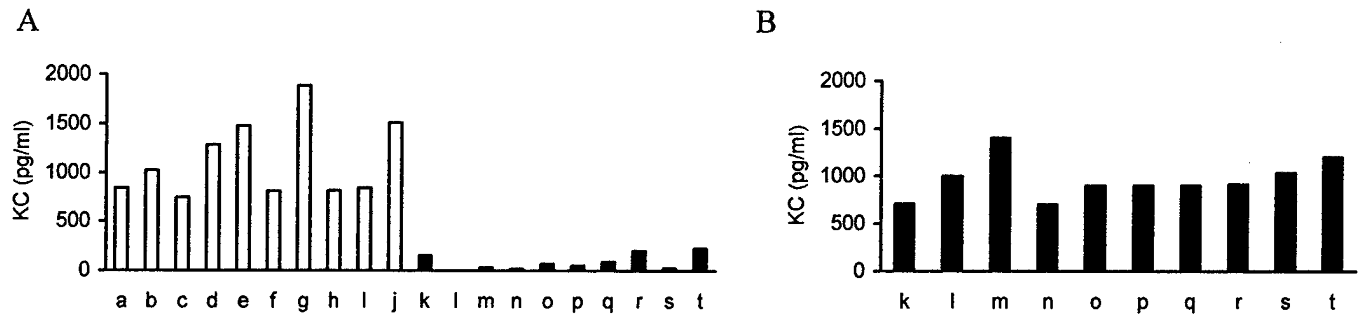

Figure 13. Screening of anti-sense transfected 4T1. A. More than 300 anti-sense transfected clones were screened by ELISA. Lanes a-j represents the vast majority of the clones that were screened. Lanes k-t represents 10 of approximately 20 clones that exhibited a decrease in KC expression. B. When the clones with low levels of KC expression were grown for another week in culture the level of KC expression was significantly increased.

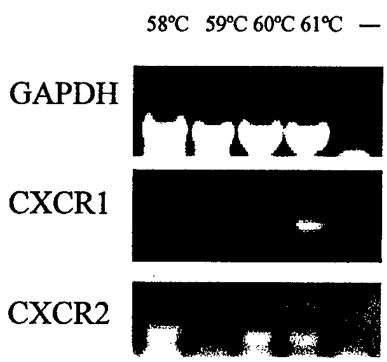

Figure 14. KC receptor expression. RT-PCR was used to determine whether the 4T1 cells expressed mRNA encoding the KC receptors; CXCR1, and CXCR2. PCR primers were designed for each and tested on a gradient cycler at the indicated annealing temperatures. GAPDH was used as a positive control. (-) indicates PCR control, no cDNA added to the reactions.

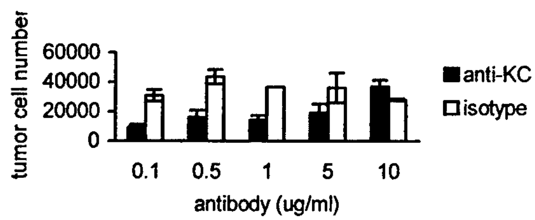

Figure 15. Antibody mediated neutralization of KC. A neutralizing antibody (R&D Systems) specific for murine KC was added at the indicated concentrations in the presence of the 4T1 cells. After 72 hours of in vitro culture the tumor cells were harvested and counted.

Summary of findings with KC

1. Tumor-derived KC can be blocked using anti-sense strategy, but the cells regain KC expression.
2. 4T1 murine mammary carcinoma cells express mRNA encoding the KC receptor.
3. Neutralization of KC using a specific antibody can slow the growth of the 4T1 murine mammary carcinoma cell line in vitro.

In Progress

We are generating the RANTES/MCP-1 double construct and will analyze the T cell response in the final year of the project for completion of milestone #2 in the statement of work. We anticipate that a strong T cell response will be elicited to a tumor that lacks both RANTES and MCP-1 because individually they inhibited the T cell response. Removal of both is therefore expected to be of even greater significance. Additionally, we will continue to work with the KC neutralizing antibody to validate whether KC is a growth factor for murine mammary cancer for milestone #4 in the statement of work. If this turns out to be true this would be the first report that this chemokine functions as an autocrine growth factor for breast cancer.

KEY RESEARCH ACCOMPLISHMENTS

- Cloning of KC, MCP-1 and RANTES gene fragments into a T vector.
- Construction of sense and anti-sense expression vectors for KC, MCP-1 and RANTES.
- Generation of stable sense and anti-sense RANTES expressing tumor cells.
- RANTES portion of project complete and manuscript in preparation. We have confirmed that RANTES modulates T cell anti-tumor immunity.
- Generation of stable sense and anti-sense MCP-1 expressing tumor cells.
- MCP-1 portion of project complete and manuscript submitted for publication. We have confirmed that MCP-1 modulates T cell anti-tumor immunity.
- Preliminary data that KC acts as an autocrine growth factor for breast cancer.

REPORTABLE OUTCOMES

1. Data generated from this project were presented at the 78th annual Pennsylvania Academy of Science (PAS) meeting. title: Blockade of the Tumor-Derived RANTES and the Impact on T-Cell Migration, Evan Adler and Robert A. Kurt, April 5-7, 2002.

2. Data generated from this project were presented at the 2002 Department of Defense Era of Hope meeting. title: Inhibition of tumor-derived MCP-1 and anti-tumor immunity, Robert A. Kurt, Erin Allison, Mara Shainheit, and Peter Vitiello, Sept 25-28, 2002.

3. Data generated from this project were presented at the 79th annual PAS meeting. Title: Investigating the Role of Tumor-Derived KC in Murine Breast Cancer, Rachel Harris and Robert A. Kurt, April 2003.

4. Data from this project were presented at the American Association of Immunologists meeting. title: Enhancement of Anti-Tumor Immunity by Inhibition of Tumor-Derived CCL5, Robert A. Kurt, Evan Adler and Nicholas Katchen, May 2003.

5. Data from this project were submitted for publication. title: Impact of tumor-derived CCL2 on T cell effector function, Robert A. Kurt, Mara G. Shainheit, Erin M. Allison, Evan P. Adler, and Peter F. Vitiello. Submitted Immunological Letters.

6. Manuscript with RANTES data generated in this project is in preparation for submission. title: Enhancement of Anti-Tumor Immunity by Inhibition of Tumor-Derived CCL5, Evan P. Adler, Nicholas Katchen, and Robert A. Kurt.

7. A grant was submitted (October 2002) to the Komen Foundation using data generated from this project. title: Blockade of Chemokine Receptor Signaling and Reversal of Tumor-Induced Immune Suppression.

8. A grant was submitted (May 2003) to the Department of Defense using data generated from this project. title: The Role of CCL2 and CCL5 in Breast Cancer Invasion and Metastasis.

CONCLUSIONS

We have hypothesized that the constitutive expression of chemokines can impair anti-tumor immunity. In order to test this hypothesis a tumor cell line that does and does not express chemokines was necessary. Using anti-sense technology we were able block RANTES and MCP-1 expression from tumor cells that normally constitutively produce these chemokines. We have found that RANTES and MCP-1 each exert a negative influence upon the tumor-specific T cell response to cancer. Studies are under way to determine the extent to which inhibition of both MCP-1 and RANTES can enhance anti-tumor immunity.

The generation of a tumor cell clone that lacks KC expression has proven more difficult. However, the difficulty posed has led to some exciting preliminary evidence that KC acts as an autocrine growth factor for the murine mammary carcinoma 4T1. If this turns out to be true these data would suggest that inhibition of chemokine expression and/or signaling may prevent down-regulation of the T cell response to cancer and at the same time directly inhibit tumor growth.

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